

# Preface

Fungi have been under a microscope almost since its inception (Zacharias and Hans Jansen 1595), with Hooke (1665) first describing and illustrating *Phragmidium mucronatum* (parasitic rose rust) and the saprophytic *Mucor*, and Malpighi (1675, 1679) documenting a variety of fungi. Every subsequent development in molecular tagging and microscopic instrumentation has impacted mycology. Since 2008, the Nobel Prize in Chemistry has been awarded twice to researchers who have developed advanced tools for microscopic imaging. The most recent was awarded last year for developing super-resolution fluorescence microscopy, which collectively pushed microscopic resolution beyond the diffraction limit, the holy grail of optical microscopy since first defined by Ernst Abbé in 1873. The second involved the discovery and development of the green fluorescent protein (GFP) which has produced a spectral rainbow of fluorescent proteins for tagging and tracking molecules in living cells. This development, revolutionized biological microscopy, and inspired the discovery of mEos fluorescent proteins which have enhanced certain types of super-resolution microscopy. Such advances now allow us to image cells at resolutions that are an order of magnitude better than diffraction limited optical approaches.

This volume is a compilation of the principles underscoring various advanced microscopy methods and how they have been, or have the potential to be, applied to mycology. Chapter 1 begins with Drs. Rosa Mouriño Perez (Centro de Investigación Científica y de Educación Superior de Ensenada) and Robby Roberson (Arizona State University) offering a comprehensive overview of the confocal principle, confocal laser scanning microscopy and its application to fungal biology. In some ways, this chapter is a cornerstone of the volume, as so many of the newer sophisticated fluorescence-based methods depend on this technology. Drs. Norio Takeshita from Karlsruhe Institute of Technology and Oier Etxebeste at the University of Basque Country teamed up in Chapter 2 to introduce us to fluorescence-based techniques that have been used to study fungi, including bimolecular fluorescence complementation (BiFC), the so-called four-letter F-words (i.e. FRET, FRAP, FLIM), and novel dyes (mEos) that have been developed for super-resolution methods. This leads us directly to Chapter 3 in which Drs. James Dodgson, Rafael Carazo Salas, Anatole Chessel from the University of Cambridge and Dr. Susan Cox at King's College London describe the various types of recently developed super resolution

microscopy techniques, the subject of this year's Nobel Prize, and how they have been used to uncover minute details within fungi both spatially and temporally. In Chapter 4, Dr. Annette Naumann from the Julius-Kühn Institute describes how Fourier transform infrared (FTIR) microscopy is uniquely poised to image and determine the chemical make-up of fungi alone, and in the context of a common substrate, wood. Chapter 5 turns to Drs. Zhiting Liang, Yong Guan, Shan Chen and Yangchao Tian at the National Synchrotron Radiation Laboratory in the University of Science and Technology in China who describe how full-field hard X-ray tomography has been applied for the first time to reconstruct, at the nanoscale, the three-dimensional (3D) structure of yeast, along with the future potential of this method. Chapter 6 by Dr. Yajing Shen from the City University of Hong Kong outlines a completely novel and clever method for the *in situ* characterization of yeast at the nano scale, using environmental scanning electron microscopy (ESEM) and focused ion beam milling (FIB). Some of the methods from Chapter 6 could also find application to atomic force microscopy (AFM), the subject of Chapter 7 by Drs. Cécile Formosa and Etienne Dague from the Centre National de la Recherche Scientifique. In this chapter, Formosa and Dague tell us how AFM can be used to image and quantify the biophysical properties of live yeast and fungi. Finally in Chapter 8, coeditors Dr. Tanya Dahms from the University of Regina and Dr. Kirk Czymmek Carl Zeiss Microscopy, Inc. describe advances in biosensors, 3D imaging, correlative microscopy, and other recent advances in microscopy methods as applied to fungi.

While most of the methods described in this volume have, at least in principle, mycological applications, there are so many open questions that could be answered using the advanced microscopy described herein. Even for microscopes that are commercialized (confocal, FTIR, AFM), they often remain most effective in the hands of microscopy experts, while other more specialized methods are usually only found in a handful of labs (super resolution and ESEM-FIB) or require a trip to a synchrotron source (X-ray tomography). That being said, those researchers who operate or create specialized instrumentation in their lab are always keen to collaborate (instruments seeking applications) and so I would encourage you to make friends with one or more microscopy specialist. Just like correlative microscopy, the fruit of collaborative research is more like the fruit tree rather than simply a collection of fruit.

## In Gratitude

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